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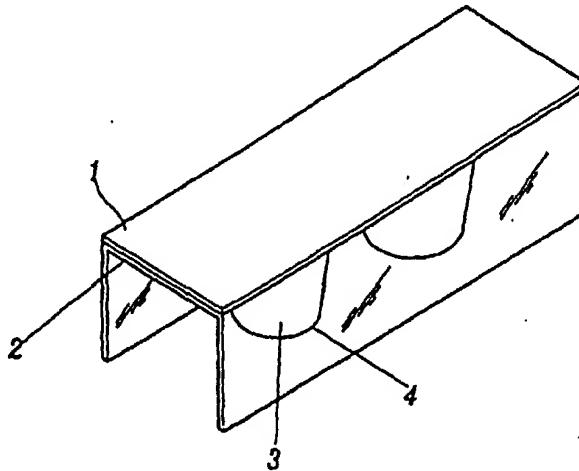
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: <b>C12Q 1/58, 1/04</b>	A1	(11) International Publication Number: <b>WO 99/51769</b> (43) International Publication Date: 14 October 1999 (14.10.99)
(21) International Application Number: <b>PCT/KR99/00161</b>	(81) Designated States: CN, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 2 April 1999 (02.04.99)	Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(30) Priority Data: 1998/11909 3 April 1998 (03.04.98) KR		
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(54) Title: COMPOSITION, KIT, AND METHOD FOR DETECTING HELICOBACTER PYLORI IN BIOPSY

## (57) Abstract

Disclosed are a composition for detecting *Helicobacter pylori* including urea 0.5 to 4 percent by volume, potassium phosphate 0.05 to 0.2 percent by volume, phenate reagent solution 0.8 to 1.7 percent by volume, an indicator having a  $pK_a$  of 6.5 to 8.5, 0.002 to 0.005 percent by volume, and a balance of water, a kit for detecting *Helicobacter pylori* using the composition and detecting method using the composition can determine quickly and accurately whether or not an infection by *Helicobacter pylori* exists, can obtain the same determination results after an elapse of time and can be used easily in an endoscope chamber.



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## COMPOSITION, KIT, AND METHOD FOR DETECTING HELICOBACTER PYLORI IN BIOPSIE

### BACKGROUND OF THE INVENTION

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#### **(a) Field of the Invention**

This invention relates to detection of Helicobacter pylori (hereinafter called H. pylori), source bacterium of gastrointestinal disorders, and in particular relates to an H. pylori detecting composition produced by applying a phenate 10 method for ammonium ion's quantitative determination to a conventional urease enzyme test and relates to a detecting kit and method using this composition.

#### **(b) Description of the Related Art**

H. pylori is noted as a source bacterium causing gastrointestinal 15 disorders including gastric cancer and gastroduodenal ulcers, and the WHO has prescribed H. pylori as a typed carcinogen. Precise diagnosis, i.e., accurate detection of H. pylori, should be promoted to cure gastrointestinal disorders caused by this H. pylori. H. pylori detecting methods being used up to now can be divided mainly into two types, i.e., non-invasive tests not using an 20 endoscope and invasive tests using an endoscope to get biopsy tissue for detection purposes.

Non-invasive tests include serology tests and  $^{13}\text{C}$  or  $^{14}\text{C}$  Breath tests. Serology tests measuring H. pylori antibodies have a problem in that the existence of the antibody does not necessarily mean that diseases are 25 developing because it takes more than 6 months for antibody levels to drop after the cure of diseases. The  $^{13}\text{C}$  or  $^{14}\text{C}$  Breath test uses the principle that if urea marked with  $^{13}\text{C}$  or  $^{14}\text{C}$  is ingested, it is transformed into marked  $\text{CO}_2$  in the stomach if H. pylori exists and detected in exhaled air. However, the method is not usually used because it involves high cost equipment.

30 On the other hand, invasive tests are more commonly used to detect H. pylori. Invasive tests detecting methods using biopsy tissues samples collected during an endoscoping includes histology, the PCR (Polymerase

Chain Reaction) method, culturing, and the urease enzyme test. Histology is a method confirming the existence of *H. pylori* through general tissue research, the PCR method is a method confirming the existence of *H. pylori* by using chain reaction of polymerization enzyme, and culturing is a method of directly 5 cultivating *H. pylori* from biopsy tissue. However, there is a problem in that all these methods are difficult and take much time, so they can not be used commercially.

The urease enzyme test, which can be used easily in the endoscope chamber and is the most efficient method, uses the fact that *H. pylori* produces 10 urease enzyme which has a much higher degree of activity compared to other microorganisms. That is, if urease exists urea added in the detecting kit decomposes and ammonia is produced to cause pH to be increased making a pH indicator react and change color. A *H. pylori* detecting method measures the activity degree of urease like this. Commercialized kits using the urease 15 enzyme test now are "CLOtest" (USP No. 4,748,113), "Hpfast" (USP No. 5,439,801) and "Pyloritek" (USP No. 5,314,804 and 5,420,016). Compositions used in "CLOtest" include 10 ~ 40 g/l of urea, 1 ~ 5 g/l of bactericide, an available quantity of  $pK_a$  6.5 ~ 8.5 indicator (phenol red) and the remainder being water, having a 5.0 ~ 6.5 pH. However, the CLOtest has problems in 20 that the positive rate vary with the readers because the reaction speed of urease that the above compositions and *H. pylori* produce is slow. Therefore, diagnosis is possible only after about 24 hours and it is not possible to obtain diagnostic results on the endoscope examination date which requires that the patient inconveniently visit the hospital once more. Furthermore, the various 25 colors appearing after more than 24 hours makes accurate diagnosis difficult. Hpfast is fundamentally similar to the CLOtest, but different in that cell wall detergent is added and uses as an indicator mixture of phenol red, methyl red, and bromothymol blue. With Hpfast, an *H. pylori* positive is determined from a dark green color of pH 6.2 and a light green color of pH 6.0 is determined as an 30 *H. pylori* negative. However, the accurate reading of this dark green and light green colors is difficult. Pyloritek uses a multilayer test device differently from

the above mentioned CLOtest and Hpfast, the main characteristics of this is that the urease reacting part and reacted product as an ammonia detecting part are on different layers and the pHs of each layers are different. Although Pyloritek can produce diagnosis results within one hour, the false positive rate 5 increases if the determination time exceeds one hour. Therefore, there is an increasing possibility of false positives if an accurate reaction time is not adhered to during the endoscope examination.

Moreover, the above mentioned urease enzyme methods including the CLOtest, Pyloritek, etc. could also react with the small amounts of low activity 10 urease which bacilli such as *Staphylococcus hominis*, *Streptococcus salivarius*, *E. aerofaciens*, *L. fermentum*, etc. have and false positive rates could be increased in cases of long decision times.

#### SUMMARY OF THE INVENTION

15 The present invention is designed to solve the above mentioned problems of the conventional technology by providing a *H. pylori* detecting composition, a *H. pylori* kit, and a method for using the above composition which allows the rapid and accurate determination of whether or not a *H. pylori* infection exists, which is the source bacterium that causes gastrointestinal 20 disorders. The present invention also provides the same test results after a period of time passed, and it can easily be used in the chamber of an endoscope.

In order to achieve the above described purpose of this invention, this 25 invention first provides *H. pylori* detecting composition including urea from 0.5 to 4 vol%,  $\text{KH}_2\text{PO}_4$ , from 0.05 to 0.2 vol%, phenate reagent solution from 0.8 to 1.7 vol%, an indicator from 0.002 to 0.005 vol% having a  $\text{pKa}$  of from 6.5 to 8.5 and a balance of water. Among the above described constituents, said phenate 30 reagent solution from 0.8 to 1.7 vol% is preferably composed of manganous sulfate solution from 0.5 to 1 vol%, hypochlorite reagent from 0.2 to 0.5 vol% and phenate reagent from 0.1 to 0.2 vol % and the above described indicator is preferably phenol red. Moreover, said composition more preferably comprises

gelling agent from 0.5 to 2 vol%. Especially said composition most preferably comprise 2 vol% urea, 0.05 vol%  $\text{KH}_2\text{PO}_4$ , 1 vol% manganous sulfate solution, 0.5 vol% hypochlorite reagent, 0.2 vol% phenate reagent, 0.0025 vol% phenol red, 1 vol% agar and a balance of water. And said composition preferably has 5 pH from 6.0 to 7.8.

Second, this invention is made from the above described composition and provides an *H. pylori* detecting kit including biopsy tissue receptical test device, positive control produced by further adding 10 ~ 20 $\mu\text{l}$  of 0.1N NaOH solution to said constituents and negative control made of said constituents 10 wherein no biopsy tissue is placed.

Third, this invention provides an *H. pylori* detecting method from biopsy tissue including stages of reacting biopsy tissue with said composition and observing color change of said composition.

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#### BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of the specification, illustrate an embodiment of the invention, and, together with the description, serve to explain the principles of the invention:

Figure 1 is a perspective view of detecting kit according to this 20 invention;

Figure 2 is a sectional view of detecting kit according to this invention; and

Figure 3 is a drawing describing application example of detecting kit according to this invention.

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#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the following detailed description, only the preferred embodiment of the invention has been shown and described, simply by way of illustration of the best mode contemplated by the inventors of carrying out the invention. As will 30 be realized, the invention is capable of modification in various obvious respects, all without departing from the invention. Accordingly, the drawings and

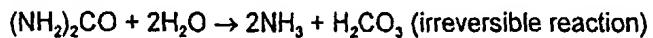
description are to be regarded as illustrative in nature, and not restrictive.

Hereinafter, further details of this invention are explained as follows:

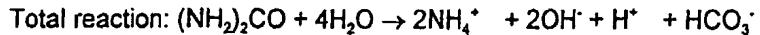
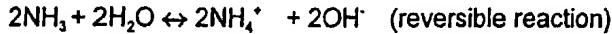
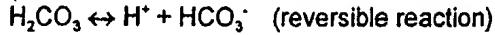
In detecting *H. pylori*, the inventors tried to substantially lower false positive rates due to the subjectivity of the readers by transforming the existing 5 urease enzyme tests from the methods conventionally used to a composition that uses a speedy change of colors that enables an accurate determination to be made rapidly by comparing the test results simultaneously with positive and negative controls. Namely, since test kits using existing urease enzyme tests detect the pH increase caused by OH<sup>-</sup> ions produced from the reaction of water 10 with the ammonia generated by simply decomposing urea, the inventors tried to eliminate the ammonium ions themselves in order to increase the rate of pH change. To do this, the inventors applied the phenate method (Standard Methods for the Examination of Water and Wastewater 19th ed. 1995, American Public Health Association, pp 4-80 ~ 4-82.) used in quantification of 15 ammonium ions.

The Urea decomposition process by urease is presented as following reaction formula:

[reaction formula 1]



20 (urea)



25 The constituents of this present invention increase pH promptly by transforming the products of the above described urea decomposition process, i.e., ammonium ions, into indophenol blue to continue urea decomposition. In order to achieve the above described purpose, the phenate method, a remarkably effective method of measuring ammonia concentration in the 30 aqueous solution, is applied. The phenate method, using the principle that ammonia under a manganic catalyst reacts with hypochlorite base and phenol

to change into indophenol blue, can be described as the following reaction formula:

[reaction formula 2]



That is, the constituents of this invention make the reaction speed faster in order to increase pH rapidly by adding phenate reagent solution to constituents used in the conventional urease enzyme test and transforming 10 ammonium ion generated in the urea decomposition process by the above described urease into indophenol blue.

Additionally, NaOCl is among the constituents that acts to reduce false positives that follow increased concentrations due to urease from bacilli as it 15 has an inhibiting effect on weak urease activity. This also allows test results to be obtained after longer testing time periods.

In this invention, the added quantity of phenate reagent solution is adjusted within a range so that *H. pylori* urease activity is not inhibited and color 20 change of the pH indicator is not affected. This invention's constituents and the possible content range of each components is as below:

urea from 0.5 to 4 vol%,  
KH<sub>2</sub>PO<sub>4</sub> from 0.05 to 0.2 vol%,  
phenate reagent solution from 0.8 to 1.7 vol%,  
25                    0.002 ~ 0.005 vol % of an indicator having pK<sub>a</sub> from 6.5 to 8.5 and  
a balance of water

From the above described composition, urea acts as a urease substrate which *H. pylori* produces, about 0.5 ~ 4 vol% of content is an appropriate level 30 to measure activity of urease which *H. pylori* produces. KH<sub>2</sub>PO<sub>4</sub> acting as a buffer solution should include 0.05 ~ 0.2 vol%, i.e., it can not obtain desired the

pH range at lower than 0.05 vol% and tends to inhibit pH change at higher than 0.2 vol%. The phenate reagent solution, acting to transform ammonium ions into indophenol blue and to inhibit urease activities of other microorganisms should be included at 0.8 ~ 1.7 vol%, i.e., at lower than 0.8 vol% the effect of increasing the pH change speed by eliminating ammonium ions and the inhibiting effect of urease activity of other microorganisms are insufficient, while at higher than 1.7 vol% pH is increased much too high and the urease activities of other microorganisms as well as *H. pylori* tend to be inhibited. An indicator having a pKa from 6.5 ~ 8.5 acts to sense pH change, with phenol red being the most appropriate in this invention, preferably between 0.002 ~ 0.005 vol% for the precise determination. Phenol red has characteristics of showing yellow in an acid solution and a purplish-red color in a basic solution.

The above described phenate reagent solution containing in detail a manganous sulfate solution acting as a catalyst, hypochlorite reagent acting as a reactant reacted with ammonium ions and phenate reagent, and is especially desirably composed of 1 vol% manganous sulfate solution, 0.5 vol% hypochlorite reagent and 0.2 vol% phenate reagent. Moreover, it is further desirable that this constituent contains 0.5 ~ 2 vol% of a gelling agent, e.g., agar, as it can then be used conveniently in the form of a soft gel. It is preferable that constituent is controlled in the range of pH 6.0 ~ 7.8 because it aides precise determination when the pH is kept in this range.

Further desirable components and contents range of this invention's constituents are as follows:

2 vol% urea, 0.05 vol%  $\text{KH}_2\text{PO}_4$ , 1 vol% manganous sulfate solution, 25 0.5 vol% hypochlorite reagent, 0.2 vol% phenate reagent, 0.0025 vol% phenol red, 1 vol% agar, and a balance of water.

On the other hand, desirable components and contents of stock solution of phenate reagent solution used in this invention are as follows:

Manganous sulfate solution; 0.05 vol%  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , and a balance of 30 distilled water,

Hypochlorite reagent; 1 vol%  $\text{NaOCl}$ , and a balance of distilled water,

Phenate reagent; 2.5 vol% NaOH, 8 vol% phenol, and a balance of distilled water.

This invention is to react with the above described constituents by placing biopsy tissue obtained through an endoscope on the gel made of the 5 above described constituents and observe color change. The gel is made of the above described constituents and a positive control gel is produced by adding to the above described constituents a minimum quantity of NaOH that can show positive results, i.e., 10 ~ 20  $\mu$ l 0.1N NaOH solution. A determination can be made as to whether or not there is an *H. pylori* infection 10 comparing the color of the negative control gel in which NaOH is not added at all. Accordingly, a test device with positive and negative controls placed side by side for comparison at a glance with colors indicating positive or negative results will decrease erroneous test results due to the subjectivity of the reader.

A perspective view of a detecting kit according to this invention is 15 presented in Figure 1 and side view in Figure 2, respectively. An application example of a detecting kit according to this invention is also presented in Figure 3. Referring to the above described Figures 1 to 3, 1 is a cover, 2 is a container, most desirably a transparent container made of an acrylic material, 3 is detecting composition according to this invention; 4 is a test device, 5 is the 20 biopsy specimen area, desirably opaquely treated such that biopsy specimen is not seen from the side, 6 is the negative control, 7 is the positive control and 8 is the biopsy sample.

A desirable practical example and comparative example of this invention are described. However, the below described practical example is 25 only one of practical examples of this invention and this invention is not limited to the below described practical example.

#### [Practical example 1]

##### Making *H. pylori* detecting compositions

30 First the below described composition of stock solution was prepared; 20 vol% urea, 2 vol%  $\text{KH}_2\text{PO}_4$ , 0.01 vol% phenol red, and 2 vol% agar.

As stock solution of phenate reagent, 100 ml of manganous sulfate solution was made by adding distilled water to  $MnSO_4 \cdot H_2O$  50 mg, 100 ml of hypochlorite reagent was made by adding distilled water to 10 ml of 10% NaOCl and the pH was adjusted to 6.8 with concentrated hypochloric acid.

5 100 ml of phenate reagent was prepared by adding distilled water to 2.5 g of NaOH and 8 ml of phenol. After the above described agar solution was autoclaved (121°C, 1.5 atm) for 15 minutes, it was left alone until it is used in the water tank at 55°C and the below described 2 X reagent were produced.

A 50 ml mixture was made by mixing the above described 10 ml urea 10 stock solution, 2.5ml  $KH_2PO_4$  stock solution, 25 ml phenol red stock solution, 1 ml manganous sulfate solution, 0.5 ml hypochlorite reagent and 0.2 ml phenate reagent and adding distilled water. After bacilli of the above described produced 2 X reagent were filtered out by 0.2  $\mu m$  filters, the same amount as 2% agar solution was mixed. As a result of that, the final concentration of the 15 constituents was as below and the final pH was 7.5 at this time:

2% urea, 0.05%  $KH_2PO_4$ , 0.0005% manganous sulfate, 0.005% NaOCl, 0.2% phenate reagent (0.005% NaOH, 0.016% phenol), 0.0025% phenol red and 1% agar.

20 [Practical example 2]

Manufacture of a H. pylori detecting kit

The composition manufactured from example 1 was injected into one wall of a multiwall plate, test device with biopsy tissue obtained from an endoscope, a positive control was made by injecting into another wall the 25 composition manufactured and by further adding 10 $\mu l$  of 0.1 N NaOH to the above described composition, a negative control was made by injecting the above described composition into another wall, thereby making an H. pylori detecting kit. The above described detecting kit is called "PET (Pylori Easy Test) kit".

## [Test example 1]

After a patient's biopsy tissue was obtained, a CLOtest and PET of example 2 were used on that biopsy tissue simultaneously, and the positive rate per hour was compared and the results were described in the below Table 1.

[Table 1]

Positive rate per hour

Hour	PET	CLOtest
1	10	3
2	10 (+0)	3 (+0)
3	13 (+3)	8 (+5)
4	13 (+0)	8 (+0)
12	No test	No test
24	13 (+0)	10 (+2)
Total positive rate	13/26	10/26 or 17/26*

10 \* 7 readings were difficult to determine.

As a result, PET could confirm 76.9% of total positives within one hour and 100 % after 3 hours. On the other hand, CLOtest could confirm only 17.6 ~ 30 % of the positives and 100% only after 24 hours. However, uncertain color which could not be distinguished distinctly appeared after 24 hours and 15 the positive rate was varied from 38.4 % (10/26) to 65.3 % (17/26) depending on the observers. Therefore, we can see that rapid determination is possible in the case where PET is used according to this invention.

After that, we observed the degree of consistency of PET results with the PCR method. Here, the PCR method used an amplifying method of 26kDa 20 protein gene, a method known as the most unique and sensitive in *H. pylori* detection (Ho, S, et al. 1991, Direct Polymerase Chain Reaction Test for detection of *H. pylori* in Human and Animals, J. Clin. Microbiol, 29 pp 2543 ~ 2549). The results were described the following table 2:

## [Table 2]

## Consistency ratio with PCR method

5

PET	CLOtest
26/26 (100 %)	16/26 (61.5 %): two false negatives, five false positives
	21/26 (80.7 %): when unreadable colors were judged to be negatives

From the above described table 2, we can see that all of the 26 biopsy tissues are consistent with the results of the PCR method when using this PET invention and that the consistency ratio ranges between 61.5 ~ 80.7% for the 10 Clotest. Therefore, we can see that it is possible to judge that this PET invention is more precise than Clotest.

## [Test example 2]

Biopsy tissue obtained during an endoscoping was refrigerated at -20 15 °C for 1 week, and the PET and CLOtest were then performed according to the same method as in test example 1 with the results compared and presented in table 3 and table 4.

## [Table 3]

20 Positive rate per hour

Hour	Practical example 1	CLOtest
1	6	1
2	11 (+5)	2 (+1)
3	13 (+2)	4 (+2)

4	13 (+0)	8 (+4)
12	13 (+0)	12 (+0)
24	13 (+0)	12 (+0)
Total positive rate	13/22	12/22*

\* 6 readings were difficult to determine.

[Table 4]

Consistency with PCR

PET	CLOtest
	21/22 (95.5%); one false negative
22/22 (100 %)	18/22 (81.8 %); when unreadable colors were judged to be negatives

5

From table 3 and table 4, if PET according to this invention is used, it is notable that precise judgement is possible on biopsy tissue in which urease activity of *H. pylori* was decreased to certain degree.

This invention could provide *H. pylori* detecting compositions which tell 10 promptly and precisely whether or not an *H. pylori* infection (the source bacterium causing gastrointestinal disorders) exists, and the same results can be obtained even after time has elapsed and the compositions are easily used in an endoscope chamber. The invention provides an *H. pylori* detecting kit and a method of using *H. pylori* detecting compositions.

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[Test example 3]

Urease decomposition capacity was examined on *H. pylori* bacterium and *Staphylococcus hominis* bacterium separated from biopsy tissue obtained during an endoscoping in order to examine the *H. pylori* peculiarity of PET.

The examination method involved first having *H. pylori* bacterium and *S. hominis* bacterium cultivated and diluted in sterilized distilled water so as to have an appropriate number of bacilli, and then PET and CLOtest were performed and compared according to the same method as in test example 1.

5 The results were described in table 5.

[Table 5]

Peculiarity comparison on *H. pylori* bacterium and *S. hominis* bacterium

Classification		UBPH (0.02 %)	CLOtest
<i>H. pylori</i>	$10^3$ cells	< 30 min	within 30 min
	$10^4$ cells	1 h	2 h
	$10^3$ cells	No response even after 24 hours	No response even after 24 hours
<i>S. hominis</i>	$10^7$ cells	8 h	6 h
	$10^8$ cells	No response even after 24 hours	10 h

10 From the above described table 5 it is noted that if PET according to this invention is used, high peculiarity detection is possible with urease activity of *H. pylori* showing a fast response and urease activity of other bacilli showing low response.

15 Although preferred embodiments of the present invention have been described in detail hereinabove, it should be clearly understood that many variations and/or modifications of the basic inventive concepts herein taught which may appear to those skilled in the pesent art will still fall within the spirit and scope of the present invention, as defined in the following claims.

WHAT IS CLAIMED IS:

1. A composition for detecting *Helicobacter pylori* including:
  - a) urea from 0.5 to 4 vol%;
  - b)  $\text{KH}_2\text{PO}_4$  from 0.05 to 0.2 vol%;
  - c) phenate reagent solution from 0.8 to 1.7 vol%;
  - d) an indicator having a  $\text{pK}_a$  of 6.5 to 8.5, 0.002 to 0.005 vol%; and
  - e) a balance of water.
- 5 2. The composition of claim 1 wherein said phenate reagent solution from 0.8 to 1.7 vol% of said *Helicobacter pylori* detecting composition comprises:
  - 10 a) manganous sulfate solution from 0.5 to 1 vol%;
  - b) hypochlorite reagent from 0.2 to 0.5 vol%; and
  - c) phenate reagent from 0.1 to 0.2 vol%.
3. The composition of claim 1 wherein said composition is a *Helicobacter pylori* detecting composition in which the indicator is phenol red.
- 15 4. The composition of claim 1 wherein said composition is a *Helicobacter pylori* detecting composition further including gelling agent from 0.5 to 2 vol%.
5. The composition of claim 4 wherein said composition is a *Helicobacter pylori* detecting composition including:
  - 20 a) 2 vol% urea;
  - b) 0.05 vol%  $\text{KH}_2\text{PO}_4$ ;
  - c) 1 vol% manganous sulfate solution;
  - d) 0.5 vol% hypochlorite reagent;
  - e) 0.2 vol% phenate reagent;
  - f) 0.0025 vol% phenol red;
  - 25 g) 1 vol% agar; and
  - h) a balance of water
6. The composition of claim 1 wherein said composition is a *Helicobacter pylori* detecting composition having a pH from 6.0 to 7.8.
7. A kit for detecting *Helicobacter pylori* including:
  - 30 a) test device made of composition according to claim 4,  
wherein biopsy tissue is placed;

15

- b) positive control manufactured by further adding 10 ~ 20  $\mu$ l of 0.1 N NaOH solution to composition of claim 4; and
- c) negative control manufactured from composition of claim 4, where biopsy tissue is not placed.

5 8. A method of detecting *Helicobacter pylori* from biopsy tissue comprising the steps of:

- a) reacting biopsy tissue and said composition according to claim 1; and
- b) observing the color change of the said biopsy tissue reacted composition.

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FIG. 1

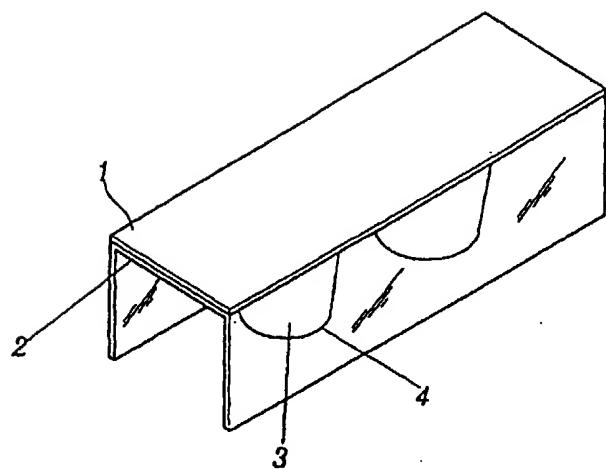
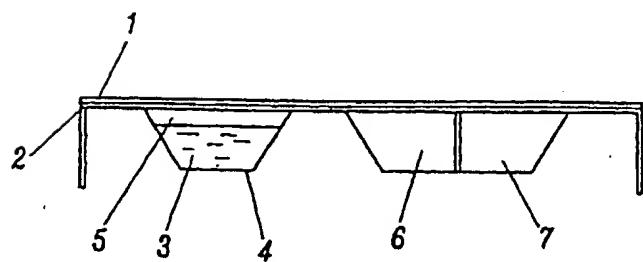
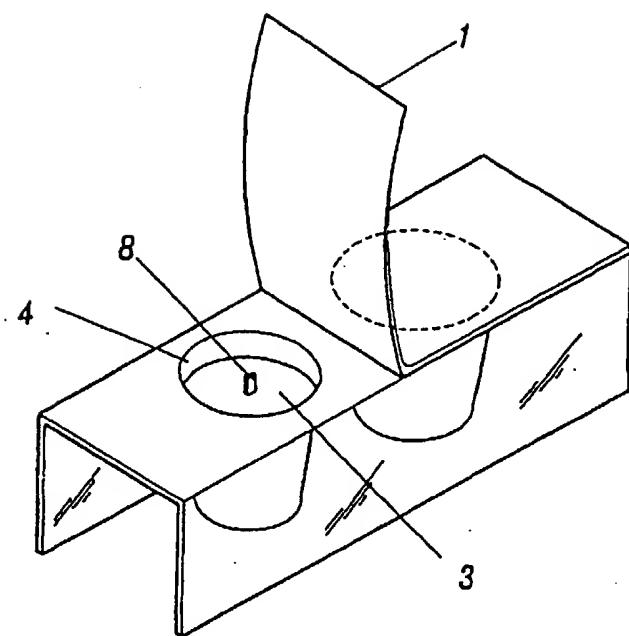


FIG. 2



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FIG. 3



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/KR 99/00161

### A. CLASSIFICATION OF SUBJECT MATTER

IPC<sup>6</sup>: C 12 Q 1/58; C 12 Q 1/04

According to International Patent Classification (IPC) or to both national classification and IPC

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC<sup>6</sup>: C 12 Q 1/58; C 12 Q 1/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAS

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 439 801 A (JACKSON), 08 August 1995 (08.08.95), column 2, lines 35-52; column 6, lines 17-21; column 7, lines 57,58; claims 1-6,9.	1,3-5,7,8
A	WO 97/30 351 A1 (THE BOOTS COMPANY PLC), 21 August 1997 (21.08.97), abstract; page 4, line 20-23; page 5, lines 2-4, 22,23; page 6, line 23- page 7, line 12; page 10, line 23 - page 11, line5.	1,2,5
A	US 5 420 016 A (BOGUSLASKI et al.), 30 May 1995 (30.05.95), column 6, lines 10-15; column 10, lines 5-12; claim 9.	1,3,7
A	EP 0 204 438 A2 (MARSHALL), 10 December 1986 (10.12.86), examples.	1,3-5,8

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents:

„A“ document defining the general state of the art which is not considered to be of particular relevance

„T“ later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

„E“ earlier application or patent but published on or after the international filing date

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„L“ document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

„Y“ document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

„O“ document referring to an oral disclosure, use, exhibition or other means

„Z“ document published prior to the international filing date but later than the priority date claimed

„&“ document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

25 June 1999 (25.06.99)

03 August 1999 (03.08.99)

Name and mailing address of the ISA/AT

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR 99/00161

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